MODIFIED RELAXIN POLYPEPTIDES COMPRISING A NON-NATURALLY ENCODED AMINO ACID IN THE A CHAIN

CROSS REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. Ser. No. 15/239,277, filed Aug. 17, 2016, now U.S. Pat. No. 9,962,450, which is a divisional of U.S. patent application Ser. No. 14/152,302, filed Jan. 10, 2014, now U.S. Pat. No. 9,452,222, which is a divisional of U.S. patent application Ser. No. 13/212,101, filed Aug. 17, 2011, now U.S. Pat. No. 8,735,539, which claims benefit to U.S. Provisional Pat. Appl. No. 61/374,582, filed Aug. 17, 2010, each of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

This application includes a sequence listing which has been submitted via EFS-Web in a file named "43270o1207.txt" created Mar. 29, 2018 and having a size of 50,306 bytes, which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

This invention relates to relaxin polypeptides optionally modified with at least one non-naturally encoded amino acid.

BACKGROUND OF THE INVENTION

Mature human relaxin is a hormonal peptide of approximately 6000 daltons known to be responsible for remodelling the reproductive tract before parturition, thus facilitating the birth process. This protein appears to modulate the restructuring of connective tissues in target organs to obtain the required changes in organ structure during pregnancy and parturition. See, Hisaw, F. L., Proc. Soc. Exp. Biol. Med., 23: 661-663 (1926); Schwabe, C., et al., Biochem. Biophys. Res. Comm., 75: 503-570 (1977); James, R. et al., Nature, 267: 544-546 (1977). A concise review of relaxin was provided by Sherwood, D. in The Physiology of Reproduction, Chapter 16, "Relaxin", Knobil, E. and Neill, J., et al. (eds.), (Raven Press Ltd., New York), pp. 585-673 (1988). Circulating levels of relaxin are elevated for the entire nine months of pregnancy and drop quickly following delivery.

While predominantly a hormone of pregnancy, relaxin has also been detected in the non-pregnant female as well as in the male. Bryant-Greenwood, G. D., Endocrine Reviews, 3: 62-90 (1982) and Weiss, G., Ann. Rev. Physiol., 46:43-52 (1984) and has most recently been found to be useful in the treatment of heart failure.

Heart failure is defined as the inability of the cardiac pump to move blood as needed to provide for the metabolic needs of body tissue. Decreases in pumping ability arise most often from loss or damage of myocardial tissue. As a result, ventricular emptying is suppressed which leads to an increase in ventricular filling pressure and ventricular wall stress, and to a decrease in cardiac output. As a physiological response to the decrease in cardiac output, numerous neuroendocrine reflexes are activated which cause systemic vasoconstriction, sympathetic stimulation of the heart and fluid retention. Although these reflex responses tend to enhance cardiac output initially, they are detrimental in the long term. The resulting increases in peripheral resistance increase the afterload on the heart and the increases in blood volume further increase ventricular filling pressure. These changes, together with the increased sympathetic stimulation of the heart, lead to further and often decompensating demands on the remaining functional myocardium.

Congestive heart failure, which is a common end point for many cardiovascular disorders, results when the heart is unable to adequately perfuse the peripheral tissues. According to recent estimates, there are about 4 million people in the United States diagnosed with this disease, and more than 50% of these cases are fatal within 5 years of diagnosis [Taylor, M. D. et al., Annual Reports in Med. Chem. 22, 85-94 (1987)].

Current therapy for heart failure, including congestive heart failure, focuses on increasing cardiac output without causing undue demands on the myocardium. To achieve these ends, various combinations of diuretics, vasodilators and inotropic agents are used to decrease blood volume, to decrease peripheral resistance, and to increase force of cardiac contraction. Current therapy therefore depends on balancing the effects of multiple drugs to achieve the clinical needs of individual patients, and is plagued by adverse reactions to the drugs used.

For example, diuretics decrease plasma concentrations of potassium and magnesium and increase the incidence of arrhythmias in patients receiving *digitalis*. Diuretics can potentiate the circulatory effects of nitrates through volume depletion and lead to decreases in filling pressure of the heart, cardiac output and systemic arterial pressure.

Alpha adrenergic antagonists can lead to marked falls in systemic arterial pressure that compromise coronary perfusion.

Angiotensin converting enzyme inhibitors can have similar effects on arterial pressure and additionally lead to excessive increases in plasma concentrations of potassium.

Drugs that lead to positive inotropy, such as *digitalis* and beta adrenergic antagonists, have the potential to provoke arrhythmias. In addition, *digitalis* has a narrow therapeutic index and the catecholamine analogs all tend to loose their effectiveness rapidly, due to receptor downregulation.

Thus there is a need for therapeutic agents that lead to physiologically integrated responses of arterial and venous vasodilation and cardiac inotropy, and are devoid of the disadvantages of the currently used therapeutic agents.

Relaxin has been purified from a variety of species including porcine, murine, equine, shark, tiger, rat, dogfish and human, and shows at least primary and secondary structural homology to insulin and the insulin-like growth factor, however homology between species can be quite low. In the human, relaxin is found in most abundance in the corpora lutea (CL) of pregnancy. However, specific nuclei in the brain have relaxin receptors and other nuclei contain messenger RNA for relaxin. Several nuclei with cells bearing relaxin receptors are found in the area of the hypothalamus.

Two human gene forms have been identified, (H1) and (H2). Hudson, P., et al., Nature, 301: 628-631 (1983); Hudson, P., et al., The EMBO Journal, 3: 2333-2339 (1984); and U.S. Pat. Nos. 4,758,516 and 4,871,670. Only one of the gene forms (H2) has been found to be transcribed in CL. It remains unclear whether the (H1) form is expressed at another tissue site, or whether it represents a pseudo-gene. When synthetic human relaxin (H2) and certain human relaxin analogs were tested for biological activity, the tests revealed a relaxin core necessary for biological activity as well as certain amino acid substitutions for methionine that